Human AFP ELISA Kit

Enzyme Immunoassay for the quantification of Human Alpha-fetoprotein (AFP) in serum

Catalog number: ARG80640
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**MANUFACTURED BY:**

Arigo Biolaboratories Corporation  
Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan  
Phone: +886 (3) 562 1738  
Fax: +886 (3) 561 3008  
Email: info@arigobio.com  

www.arigobio.com
INTRODUCTION

Alpha-fetoprotein (AFP) is a glycoprotein with a molecular weight of approximately 70 KD. AFP is normally produced during fetal and neonatal development by the liver, yolk sac, and in small concentrations by the gastrointestinal tract. After birth, serum AFP concentrations decrease rapidly, and by the second year of life and thereafter only trace amounts are normally detected in serum.

Elevation of serum AFP to abnormally high values occurs in several malignant diseases, most notably nonseminomatous testicular cancer and primary hepatocellular carcinoma. In the case of nonseminomatous testicular cancer, a direct relationship has been observed between the incidence of elevated AFP levels and the stage of disease. Elevated AFP levels have also been observed in patients diagnosed with seminoma with nonseminomatous elements, but not in patients with pure seminoma.

In addition, elevated serum AFP concentrations have been measured in patients with other noncancerous diseases, including ataxia telangiectasia, hereditary tyrosinemia, neonatal hyperbilirubinemia, acute viral hepatitis, chronic active hepatitis and cirrhosis. Elevated serum AFP concentrations are also observed in pregnant women. Therefore, AFP measurements are not recommended for use as a screening procedure to detect the presence of cancer in the general population.
Human AFP ELISA Kit ARG80640

PRINCIPLE OF THE ASSAY

This assay employs the sandwich quantitative enzyme immunoassay technique. An antibody specific for AFP has to be bound onto a pre-coated Streptavidin microtiter plate. Standards or samples are pipetted into the wells and any AFP present is bound by the immobilized antibody. After washing away any unbound substances, a Horseradish Peroxidase (HRP)-conjugated antibody specific for AFP is added to each well and incubate. Following a washing to remove unbound substances, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of AFP bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of AFP in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Storage information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody-coated microplate</td>
<td>12 strips x 8-well</td>
<td>4°C.</td>
</tr>
<tr>
<td>Standard 0-4 (0, 10, 40, 80, 160 IU/ml)</td>
<td>5 vials</td>
<td>4°C, Lyophilized</td>
</tr>
<tr>
<td>HRP conjugated antibody</td>
<td>11 ml (ready for use)</td>
<td>4°C (Protect from light)</td>
</tr>
<tr>
<td>TMB substrate</td>
<td>14 ml (ready for use)</td>
<td>4°C (Protect from light)</td>
</tr>
<tr>
<td>STOP solution</td>
<td>14 ml (ready for use)</td>
<td>4°C</td>
</tr>
</tbody>
</table>
MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30
minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Avoid using haemolytic, icteric or lipaemic samples.

REAGENT PREPARATION

- **Standards**: Reconstitute with 0.5 ml of distilled or deionized water and mix.
- **Samples**: If the initial assay found samples contain AFP higher than the highest standard, the samples can be diluted with Standard 0 and then re-assay the samples.
For the calculation of the concentrations this dilution factor has to be taken into account.
Example:
   a) Dilution 1:10: 10 µL Serum + 90 µL Standard 0 (mix thoroughly).
   b) Dilution 1:100: 10 µL 1:10 diluted a) + 90 µL Standard 0 (mix thoroughly).

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use.
Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 25 µl of standards, controls and samples in duplicate into sample wells.
3. Add 100 µl of HRP conjugated antibody into each well. Thoroughly mix for 10 sec. It is important to mix completely in this step.
4. Cover the wells and incubate for 30 minutes at RT.
5. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× distilled water (400 μl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add 100 μl of TMB mixture to each well. Incubate for 10 minutes at room temperature in dark.
7. Add 50 μl of Stop Solution to each well.
8. Read the OD with a microplate reader at 450 nm immediately.

**CALCULATION OF RESULTS**

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

(Conversion: 1IU/mL = 1.21ng/mL)

QUALITY ASSURANCE

Sensitivity

The analytical sensitivity was calculated from the mean plus two standard deviations of twenty (20) replicate analyses of Standard 0 and was found to be 1.78 IU/mL.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 4.24% and inter-assay precision was 5.64%.
Recovery
86.7-106.5%

Interfering Substances
Any improper handling of samples or modification of this test might influence the results. Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.